

AMENDED CLAIMS

1. An analog of bacteriocidal peptide microcin J25 (MccJ25) that (1) has an amino acid sequence that differs from that of MccJ25 in terms of at least one amino acid substitution, insertion, or deletion; and (2) that binds a bacterial RNAP and inhibits an activity of bacterial RNAP with a potency at least equal to that of MccJ25.

2. An analog according to claim 1 selected from the group consisting of [Lys₅]MccJ25, [Lys₁₃]MccJ25, [Lys₁₅]MccJ25, and [Lys₁₇]MccJ25.

3. An analog according to claim 1 selected from the group consisting of [X-Lys₅]MccJ25, [X-Lys₁₃]MccJ25, [X-Lys₁₅]MccJ25, and [X-Lys₁₇]MccJ25, where X contains a detectable group.

4. An analog according to claim 3 where the detectable group contains a chromophore.

5. An analog according to claim 3 where the detectable group contains a fluorophore.

6. An analog according to claim 3 where the detectable group is Cy3.

7. An analog according to claim 1 that also contains a detectable group.

8. An analog according to claim 7 where the detectable group contains a chromophore.

9. An analog according to claim 7 where the detectable group contains a fluorophore.

10. An analog according to claim 7 where the detectable group is Cy3.

11. A method for identifying an agent that binds to a bacterial RNAP homologous secondary channel amino acid sequence in a first entity, comprising the steps of: (a) preparing a reaction solution including the agent to be

tested and a first entity including a bacterial RNAP homologous secondary channel amino acid sequence; and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the bacterial RNAP homologous secondary channel amino acid sequence.

12. The method of claim 11 wherein the first entity is an intact bacterial RNAP.

13. The method of claim 11 wherein the first entity is a fragment of a bacterial RNAP.

14. The method of claim 11 wherein the first entity is a derivative of *Escherichia coli* RNAP.

15. The method of claim 11 wherein the first entity is a derivative of *Bacillus subtilis* RNAP.

16. The method of claim 11 further comprising comparison of: (a) the binding of the agent to the first entity; and (b) the binding of the agent to a second entity that contains a derivative of a bacterial RNAP homologous secondary channel amino acid having at least one substitution, insertion, or deletion.

17. The method of claim 16 wherein the second entity is a derivative of an intact bacterial RNAP.

18. The method of claim 16 wherein the second entity is a derivative of a fragment of a bacterial RNAP.

19. The method of claim 16 wherein the second entity is a derivative of *Escherichia coli* RNAP.

20. The method of claim 16 wherein the second entity is a derivative of *Bacillus subtilis* RNAP.

21. The method of claim 11 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the first entity; and (b) at least one of the presence,

1 PEA/US

extent, concentration-dependence, or kinetics of binding of the agent to a eukaryotic RNAP derivative.

22. The method of claim 21 wherein the eukaryotic RNAP derivative is a human RNAP derivative.

23. The method of claim 21 wherein the eukaryotic RNAP derivative is a human RNAP II derivative.

24. The method of claim 11 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the first entity; and (b) at least one of the presence, extent, concentration-dependence, or kinetics of binding of MccJ25 to the first entity.

25. A method for identifying an agent that inhibits an activity of a bacterial RNAP by binding to a bacterial RNAP homologous secondary channel amino acid sequence, comprising: (a) preparing a reaction solution comprising the agent to be tested and a first entity containing a bacterial RNAP homologous secondary channel amino acid sequence; and (b) detecting the at least one of the presence, extent, concentration-dependence, or kinetics of inhibition of an activity of said first entity, wherein inhibition involves binding of the agent to the homologous bacterial RNAP secondary channel amino acid sequence.

26. The method of claim 25 wherein the first entity is an intact bacterial RNAP.

27. The method of claim 25 wherein the first entity is a fragment of a bacterial RNAP.

28. The method of claim 25 wherein the first entity is a derivative of *Escherichia coli* RNAP.

29. The method of claim 25 wherein first entity is a derivative of *Bacillus subtilis* RNAP.

IDEA/US

75

30. The method of claim 25 wherein the activity is RNA synthesis.

31. The method of claim 25 wherein the activity is NTP uptake.

32. The method of claim 25 wherein the activity is pyrophosphate release.

33. The method of claim 25 wherein the activity is abortive-RNA release.

34. The method of claim 25 wherein the activity is edited-RNA release.

35. The method of claim 25 wherein the activity is transcriptional pausing.

36. The method of claim 25 wherein the activity is transcriptional arrest.

37. The method of claim 25 wherein the activity is Gre-factor binding.

38. The method of claim 25 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of the inhibition by the agent of an activity of the first entity; and (b) at least one of the presence, extent, concentration-dependence, or kinetics of the inhibition by the agent of an activity of a second entity that contains a derivative of a bacterial RNAP homologous secondary channel amino acid having at least one substitution, insertion, or deletion.

39. The method of claim 38 wherein the second entity is a derivative of an intact bacterial RNAP.

40. The method of claim 38 wherein the second entity is a derivative of a fragment of a bacterial RNAP.

41. The method of claim 38 wherein the second entity is a derivative of *Escherichia coli* RNAP.

42. The method of claim 38 wherein the second entity is a derivative of *Bacillus subtilis* RNAP.

43. The method of claim 38 wherein the activity is RNA synthesis.

44. The method of claim 38 wherein the activity is NTP uptake.

45. The method of claim 38 wherein the activity is pyrophosphate release.

46. The method of claim 38 wherein the activity is abortive-RNA release.

47. The method of claim 38 wherein the activity is edited-RNA release.

48. The method of claim 38 wherein the activity is transcriptional pausing.

49. The method of claim 38 wherein the activity is transcriptional arrest.

50. The method of claim 38 wherein the activity is Gre-factor binding.

51. The method of claim 38 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the agent of an activity of the first entity; and (b) at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the agent of an activity of a eukaryotic RNAP derivative.

52. The method of claim 51 wherein the eukaryotic RNAP derivative is a human RNAP derivative.

53. The method of claim 51 wherein the eukaryotic RNAP derivative is a human RNAP II derivative.

54. The method of claim 25 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by the

1 PEA/US

agent of an activity of the first entity; and (b) at least one of the presence, extent, concentration-dependence, or kinetics of inhibition by MccJ25 of an activity of the first entity.

55. A method for identifying an agent that binds to a bacterial RNAP homologous secondary channel amino acid sequence, comprising (a) preparing a reaction solution comprising the agent to be tested, a reference compound that binds to a homologous bacterial RNAP secondary channel amino acid sequence, and a first entity containing a bacterial RNAP homologous secondary channel amino acid sequence, and (b) detecting at least one of the presence, extent, concentration-dependence, or kinetics of competition by the agent for binding of the reference compound to the homologous secondary channel amino acid sequence.

56. The method of claim 55 wherein the first entity is an intact bacterial RNAP.

57. The method of claim 55 wherein the first entity is a fragment of a bacterial RNAP.

58. The method of claim 55 wherein the first entity is a derivative of *Escherichia coli* RNAP.

59. The method of claim 55 the first entity is a derivative of *Bacillus subtilis* RNAP.

60. The method of claim 55 wherein the reference compound contains a detectable group.

61. The method of claim 60 wherein the detectable group contains a chromophore.

62. The method of claim 60 wherein the detectable group contains a fluorophore.

63. The method of claim 55 wherein the reference compound is MccJ25.

64. The method of claim 55 wherein the reference compound is a MccJ25 derivative.

65. The method of claim 55 wherein the reference compound is a chromophore-labelled MccJ25 derivative.

66. The method of claim 55 wherein the reference compound is a fluorophore-labelled MccJ25 derivative.

67. The method of claim 55 wherein the reference compound is selected from the group consisting of [Cy3-Lys₅]-MccJ25 and [Cy3-Lys₁₃]-MccJ25.

68. The method of claim 55 further comprising measurement of FRET.

69. The method of claim 55 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of the binding of the agent to the first entity; and (b) at least one of the presence, extent, concentration-dependence, or kinetics of the binding of the agent to a second entity that contains a derivative of a bacterial RNAP homologous secondary channel amino acid having at least one substitution, insertion, or deletion.

70. The method of claim 69 wherein the second entity is a derivative of an intact bacterial RNAP.

71. The method of claim 69 wherein the second entity is a derivative of a fragment of a bacterial RNAP.

72. The method of claim 69 wherein the second entity is a derivative of *Escherichia coli* RNAP.

73. The method of claim 69 wherein the second entity is a derivative of *Bacillus subtilis* RNAP.

74. The method of claim 69 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the first entity, and (b) at least one of the presence,

extent, concentration-dependence, or kinetics of binding of the agent to a eukaryotic RNAP derivative.

75. The method of claim 74 wherein the eukaryotic RNAP derivative is a human RNAP derivative.

76. The method of claim 74 wherein the eukaryotic RNAP derivative is a human RNAP II derivative.

77. The method of claim 55 further comprising comparison of: (a) at least one of the presence, extent, concentration-dependence, or kinetics of binding of the agent to the first entity; and (b) at least one of the presence, extent, concentration-dependence, or kinetics of binding of MccJ25 to the first entity.